

Figure 1

NR-LU-13 Heavy chain variable region sequences

GAG GTT CAG CTG CAG CAG TCT GGG GCA GAG CTT GTG AAG CCA GGG GCC TCA GTC
Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala Ser Val 18

CDR1

AGG TTG TCC TGC ACA GCT TCT GGC TTC AAC ATT AAA GAC ACC TAT ATG CAC TGG
Arg Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Thr Tyr Met His Trp 36

CDR2

GTG ATA GAG AGG CCT GAA CAG GGC CTG GAG TGG ATT GGA AGG ATT GAT CCT GCG
Val Ile Glu Arg Pro Glu Gln Gly Leu Glu Trp Ile Gly Arg Ile Asp Pro Ala 54

AAT GGT AAT ACT AAA TGT GAC CCG AAG TTC CAG GGC AAG GCC ACT ATA ACA GCA
Asn Gly Asn Thr Lys Cys Asp Pro Lys Phe Gln Gly Lys Ala Thr Ile Thr Ala 72

GAC ACA TCC TCC AAC ACA GCC TAC CTG CAG CTC AGC AGC CTG ACA TCT GAG GAC
Asp Thr Ser Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp 90

CDR3

ACT GCC GTC TAT TAC TGT TCT AGA GAG GTC CTA ACT GGG ACG TGG TCT TTG GAC
Thr Ala Val Tyr Tyr Cys Ser Arg Glu Val Leu Thr Gly Thr Trp Ser Leu Asp 108

TAC TGG GGT CAA GGA ACC TCA GTC ACC GTC TCC TCA
Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser 120

NR-LU-13 Light chain variable region sequences

GAC ATC CAG ATG ATT CAG TCT CCA TCG TCC ATG TTT GCC TCT CTG GGA GAC AGA
Asp Ile Gln Met Ile Gln Ser Pro Ser Ser Met Phe Ala Ser Leu Gly Asp Arg 18

CDR1

GTC AGT CTC TCT TGT CGG GCT AGT CAG GGC ATT AGA GGT AAT TTA GAC TGG TAT
Val Ser Leu Ser Cys Arg Ala Ser Gln Gly Ile Arg Gly Asn Leu Asp Trp Tyr 36

CDR2

CAG CAG AAA CCA GGT GGA ACT ATT AAA CTC CTG ATC TAC TCC ACA TCC AAT TTA
Gln Gln Lys Pro Gly Gly Thr Ile Lys Leu Leu Ile Tyr Ser Thr Ser Asn Leu 54

AAT TCT GGT GTC CCA TCA AGG TTC AGT GGC AGT GGG TCT GGG TCA GAT TAT TCT
Asn Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Ser Asp Tyr Ser 72

CTC ACC ATC AGC AGC CTA GAC TCT GAA GAT TTT GCA GAC TAT TAC TGT CTA CAG
Leu Thr Ile Ser Ser Leu Asp Ser Glu Asp Phe Ala Asp Tyr Tyr Cys Leu Gln 90

CDR3

CGT AAT GCG TAT CCG TAC ACG TTC GGA GGG GGG ACC AAG CTG GAA ATA AAA
Arg Asn Ala Tyr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys 107

Figure 2

Light chain									
1				5					10
ASP	ILE	GLN	MET	THR	GLN	SER	PRO	SER	SER
11				15					20
LEU	SER	ALA	SER	VAL	GLY	ASP	ARG	VAL	THR
21				25					30
ILE	THR	CYS	ARG	ALA	SER	GLN	GLY	ILE	ARG
31				35					40
GLY	ASN	LEU	ASP	TRP	TYR	GLN	GLN	LYS	PRO
41				45					50
GLY	LYS	GLY	PRO	LYS	LEU	LEU	ILE	TYR	SER
51				55					60
THR	SER	ASN	LEU	ASN	SER	GLY	VAL	PRO	SER
61				65					70
ARG	PHE	SER	GLY	SER	GLY	SER	GLY	SER	ASP
71				75					80
TYR	THR	LEU	THR	ILE	SER	SER	LEU	GLN	PRO
81				85					90
GLU	ASP	PHE	ALA	THR	TYR	TYR	CYS	LEU	GLN
91				95					100
ARG	ASN	ALA	TYR	PRO	TYR	THR	PHE	GLY	GLN
101				105					
GLY	THR	LYS	LEU	GLU	ILE	LYS			

The humanised sequence of NRX451 light chain, residue positions which differ between NR-LU-13 & NRX451-humanised are marked with bold type.

Figure 3

Heavy chain									
1				5					10
GLN	VAL	GLN	LEU	VAL	GLN	SER	GLY	ALA	GLU
11				15					20
VAL	LYS	LYS	PRO	GLY	ALA	SER	VAL	LYS	VAL
21				25					
SER	CYS	LYS	ALA	SER	GLY	PHE	ASN	ILE	LYS
31									
ASP	THR	TYR	MET	HIS	TRP	VAL	ARG	GLN	ALA
41				45					50
PRO	GLY	GLN	GLY	LEU	GLN	TRP	MET	GLY	ARG
51									
ILE	ASP	PRO	ALA	ASN	GLY	ASN	THR	LYS	CYS
61				65					70
ASP	LEU	SER	PHE	GLN	GLY	ARG	VAL	THR	ILE
71				75					80
THR	ALA	ASP	THR	SER	ILE	ASN	THR	ALA	TYR
81				85					90
MET	GLU	LEU	SER	SER	LEU	ARG	SER	ASP	ASP
91				95					100
THR	ALA	VAL	TYR	TYR	CYS	SER	ARG	GLU	VAL
101				105					110
LEU	THR	GLY	THR	TRP	SER	LEU	ASP	TYR	TRP
111				115					120
GLY	GLN	GLY	THR	LEU	VAL	THR	VAL	SER	SER

The humanized sequence of NRX451 heavy chain, residue positions which differ between NR-LU-13 and NRX451-humanised are marked with bold type.

Figure 4

Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTIGEN FOUND BY
ANTIBODY NR-LU-13 AND THEIR USE IN PRETARGETING METHODS

Inventors: Scott S. Coyes et al. Serial No.: Not yet assigned Docket No.: 690022.527C2

Alignment of the Light Chain Variable Regions of NR-LU-13 (top) and humanized NRX451 (bottom).

```

DIQMISSPSSMFASLGDRVSLSC RASOGIRGNLD WYQOKPGGTIKLLIY STSNLNS
.....
DIQMTQSPSSLSASVGDRVTITC RASOGIRGNLD WYQOKPGKGP KLLIY STSNLNS
                        CDR1                                CDR2

```

```

GVPSRFGSGSGSGSDYSLTISSLESEDFADYYC LORNAYPYTF GGGTKLEIK
.....
GVPSRFGSGSGSGSDYTLTISSLPEDFATYYC LORNAYPYTF GQGTKLEIK
                        CDR3

```

Alignment of the Heavy Chain Variable Regions of NR-LU-13 (top) and humanized NRX 451 (bottom)

```

EVQLQQSGAELVKPGASVRLSCTASGFNIK DTYME WVIERPEQGLEWIG
.....
QVQLVQSGAEVKKPGASVKVSKASGFNIK DTYME WVRQAPGQGLQWMG
                        CDR1

```

```

RIDPANGNTK CDPKFQ GKATITADTSSNTAYLQLSSLTSED TAVYYCS
.....
RIDPANGNTK CDLSFQGRVTITADTSINTAYMELSSLRSDDTAVYYCS
      CDR2

```

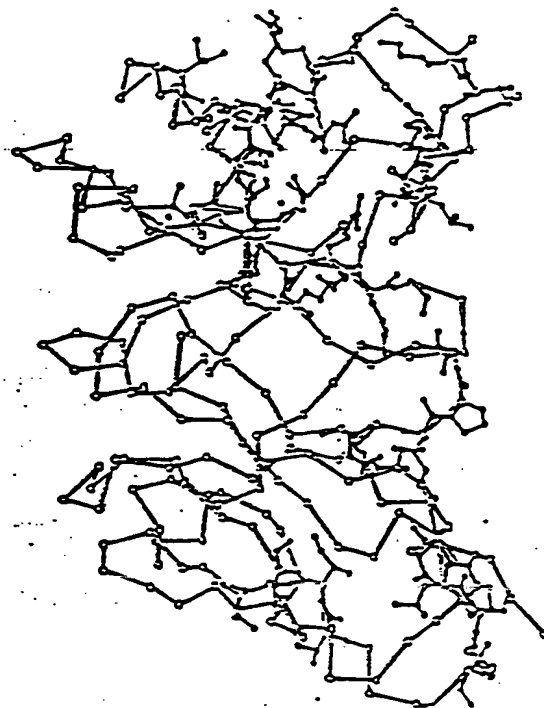
```

REVLTGTWSLDY WGQGTSVTVSS
.....
REVLTGTWSLDY WGQGTLVTVSS
      CDR3

```

Figure 5

A



B

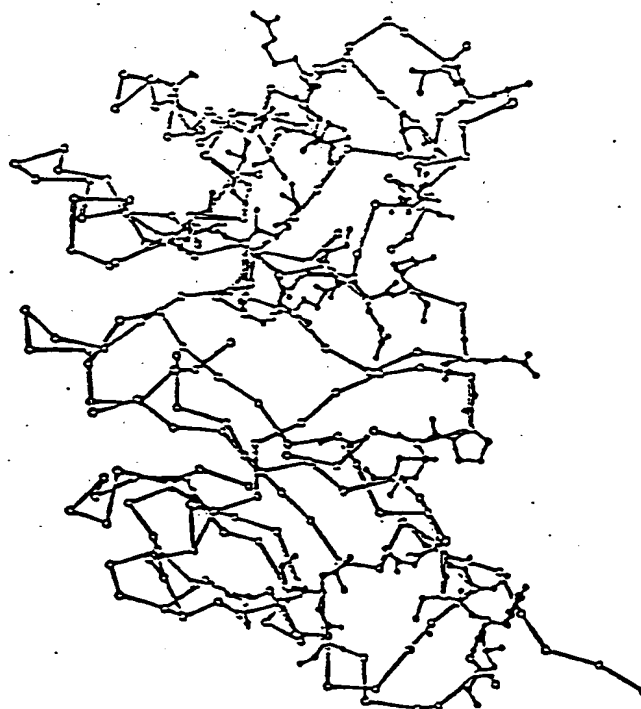


Figure 6

Same frequencies but matching with human sequences. Only one occurrence of Asp at position 182 is found and no occurrences of Cys at position 181.

RES	181	182
A	-	0.48
R	-	0.02
N	0.01	0.18
D	0.00	0.00
C	0.00	0.00
Q	0.00	-
E	-	-
G	0.00	0.01
H	0.00	-
I	-	0.00
L	-	0.00
K	0.00	0.00
M	-	-
F	0.03	-
P	0.00	0.01
S	0.01	0.23
T	-	0.02
W	0.00	-
Y	0.91	-
V	0.00	0.02
X	0.01	0.02
O	-	-
-	-	-
Z	-	-
B	-	0.00
Total	1.00	1.00

Figure 7A

Sequence positions 50 and 183 are structural mutations within 5 A of the CDR's. Frequency of residue types at these positions are identical.

Position 50 (153 human lambda sequences)

RES	50
A	-
R	-
N	-
D	-
C	-
Q	-
E	-
G	-
H	-
I	0.00
L	-
K	-
M	0.00
F	-
P	0.93
S	-
T	-
W	-
Y	-
V	-
X	0.06
O	-
-	-
Z	-
B	-
Total	1.00

Figure 7B

Position 50 (279 human kappa sequences)

RES	50
A	0.00
R	-
N	-
D	-
C	-
Q	-
E	-
G	-
H	-
I	0.00
L	0.00
K	-
M	-
F	-
P	0.96
S	-
T	-
W	-
Y	-
V	-
X	0.03
O	-
-	-
Z	-
B	-
Total	1.00

Figure 7C

Position 50 is highly conserved in all the sequences but proline can be exchanged by Ile or Leu. The framework used for the light chain (6fab) does have an Ile at this position. If this position is compared to other structures the backbone torsions are the same for structures with a Pro and an Ile at this position.

Position 183 (561 human sequences)

RES	183
A	0.06
R	-
N	0.00
D	0.21
C	-
Q	0.15
E	0.01
G	0.01
H	-
I	0.00
L	0.00
K	0.00
M	-
F	0.00
P	0.40
S	0.01
T	0.01
W	-
Y	0.00
V	0.08
X	0.02
O	-
-	-
Z	-
B	0.00
Total	1.00

Figure 7D

Position 183 (1210 mouse sequences)

RES	183
A	0.16
R	0.00
N	0.00
D	0.13
C	-
Q	0.16
E	0.25
G	0.02
H	0.00
I	-
L	-
K	0.00
M	-
F	-
P	0.17
S	0.08
T	0.00
W	-
Y	-
V	0.00
X	0.02
O	-
-	-
Z	-
B	-
Total	1.00

Leu is seen in human sequences at this position, but never in murine sequences, for both human and murine Sequences P is the most frequently occurring residue at position 183.

Figure 7E

Comments for pcDNA3:
5446 nucleotides

CMV promoter: bases 209-863

T7 promoter: bases 864-882

Polylinker: bases 889-994

Sp6 promoter: bases 999-1016

BGH poly A: bases 1018-1249

SV40 promoter: bases 1790-2115

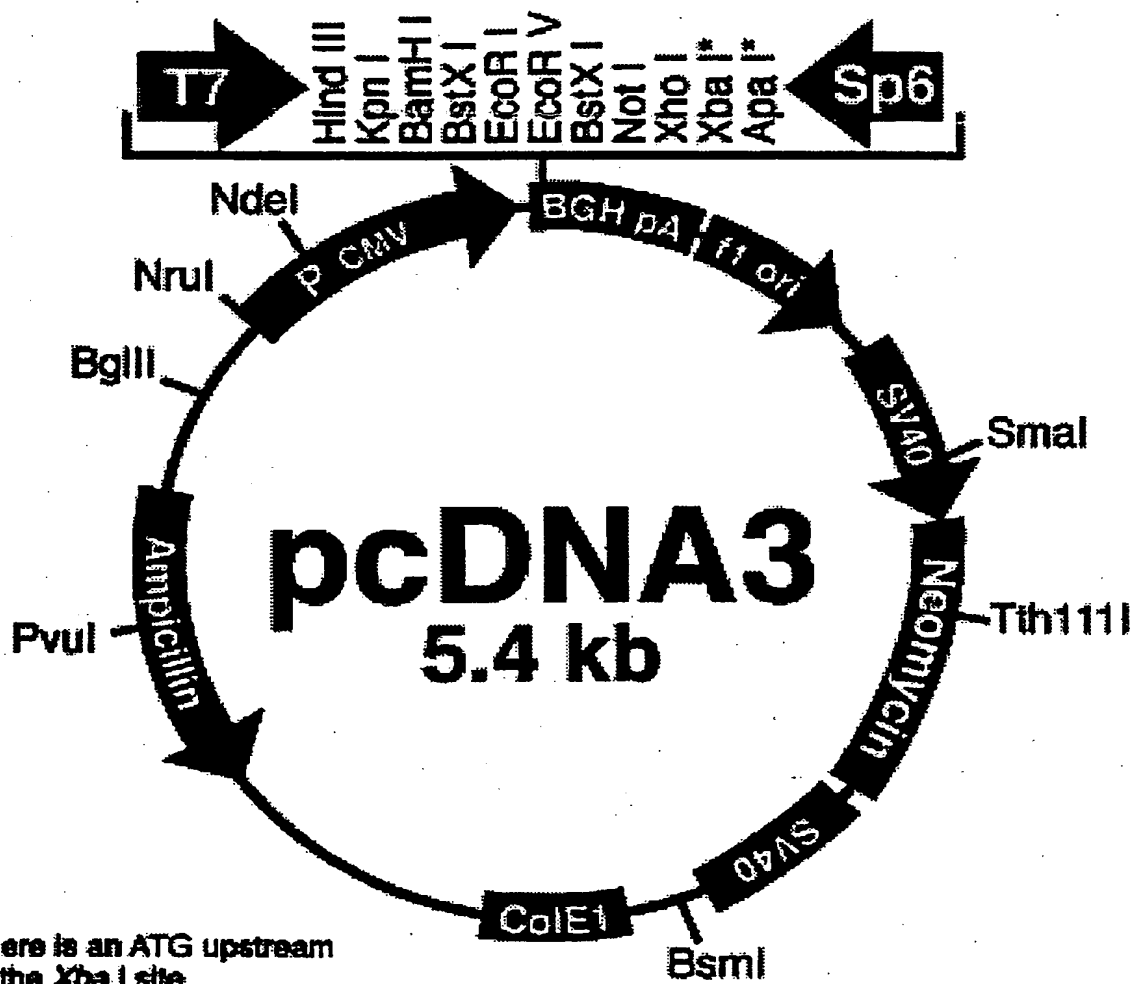
SV40 origin of replication: bases 1984-2069

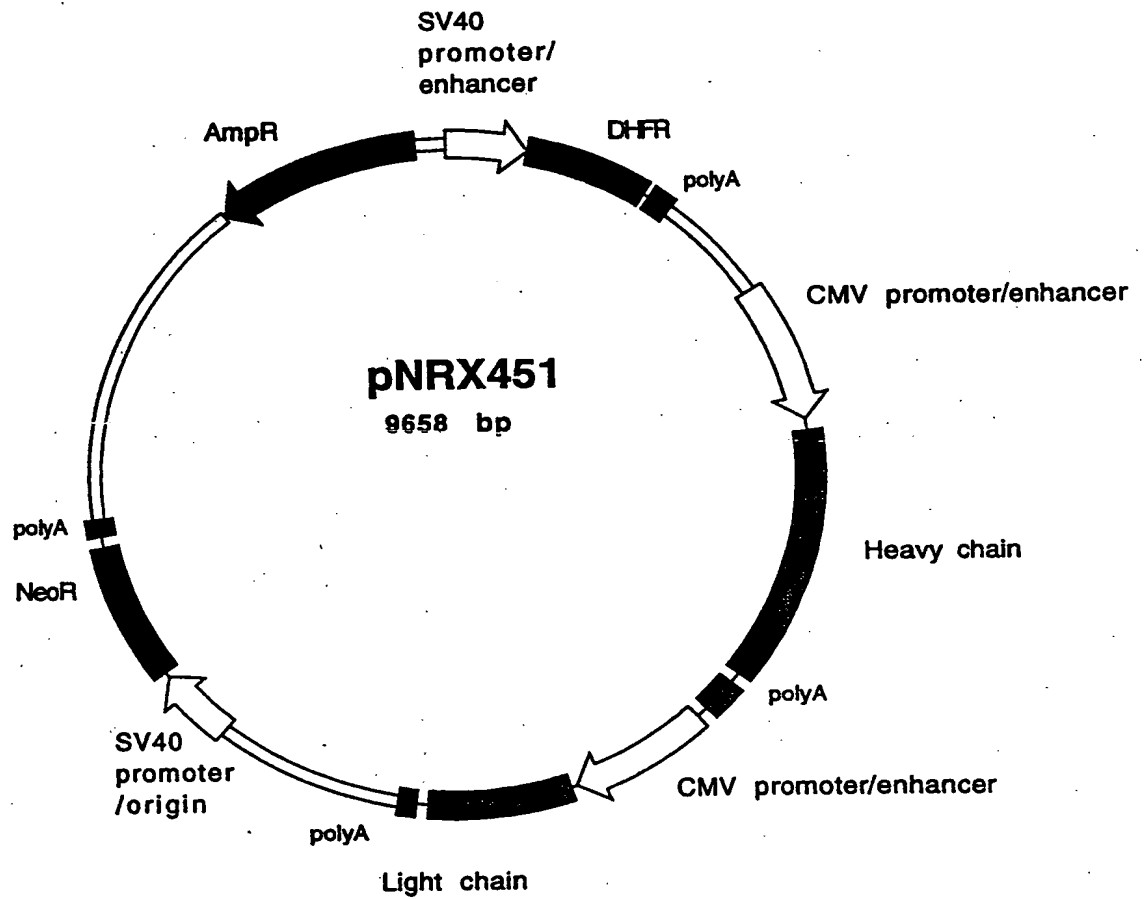
Neomycin ORF: bases 2151-2945

SV40 poly A: bases 3000-3372

ColE1 origin: bases 3632-4305

Ampicillin ORF: bases 4450-5310

**Figure 8**

**Figure 9**

POOL OF CLONE

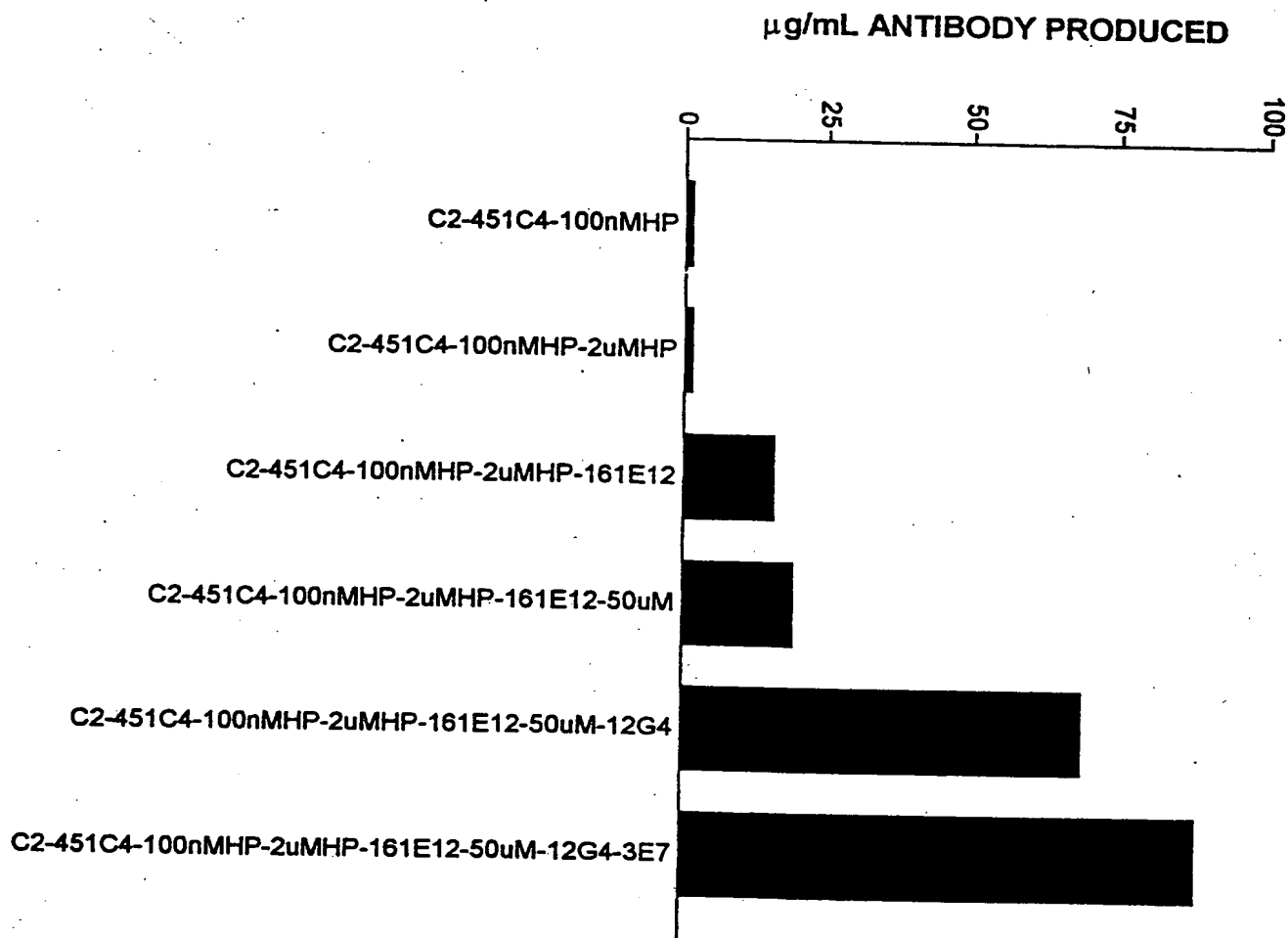
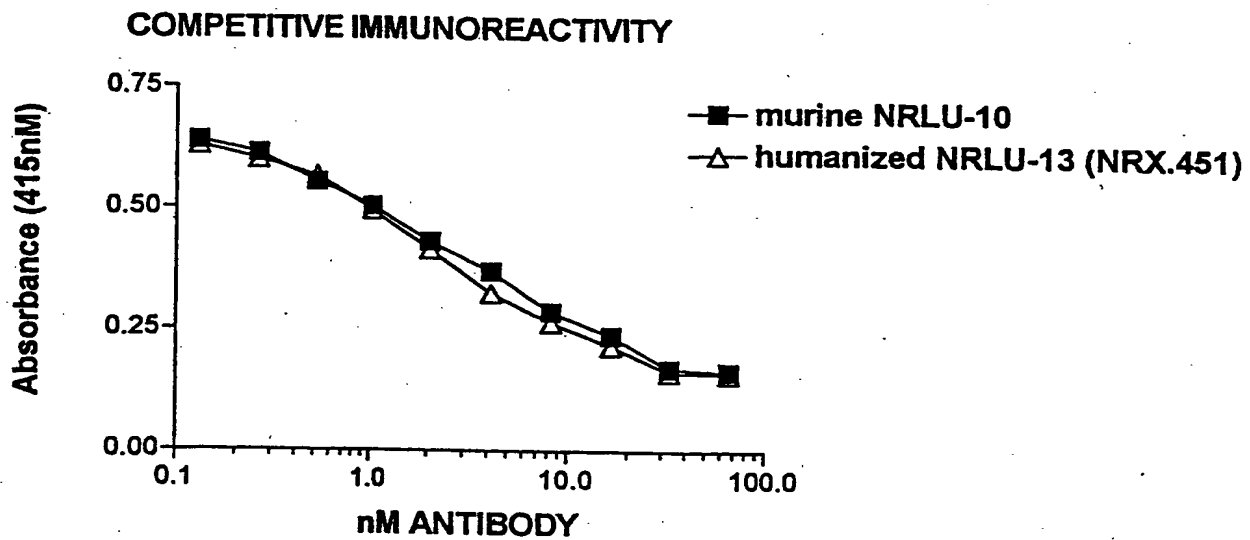


Figure 10

**Figure 11**

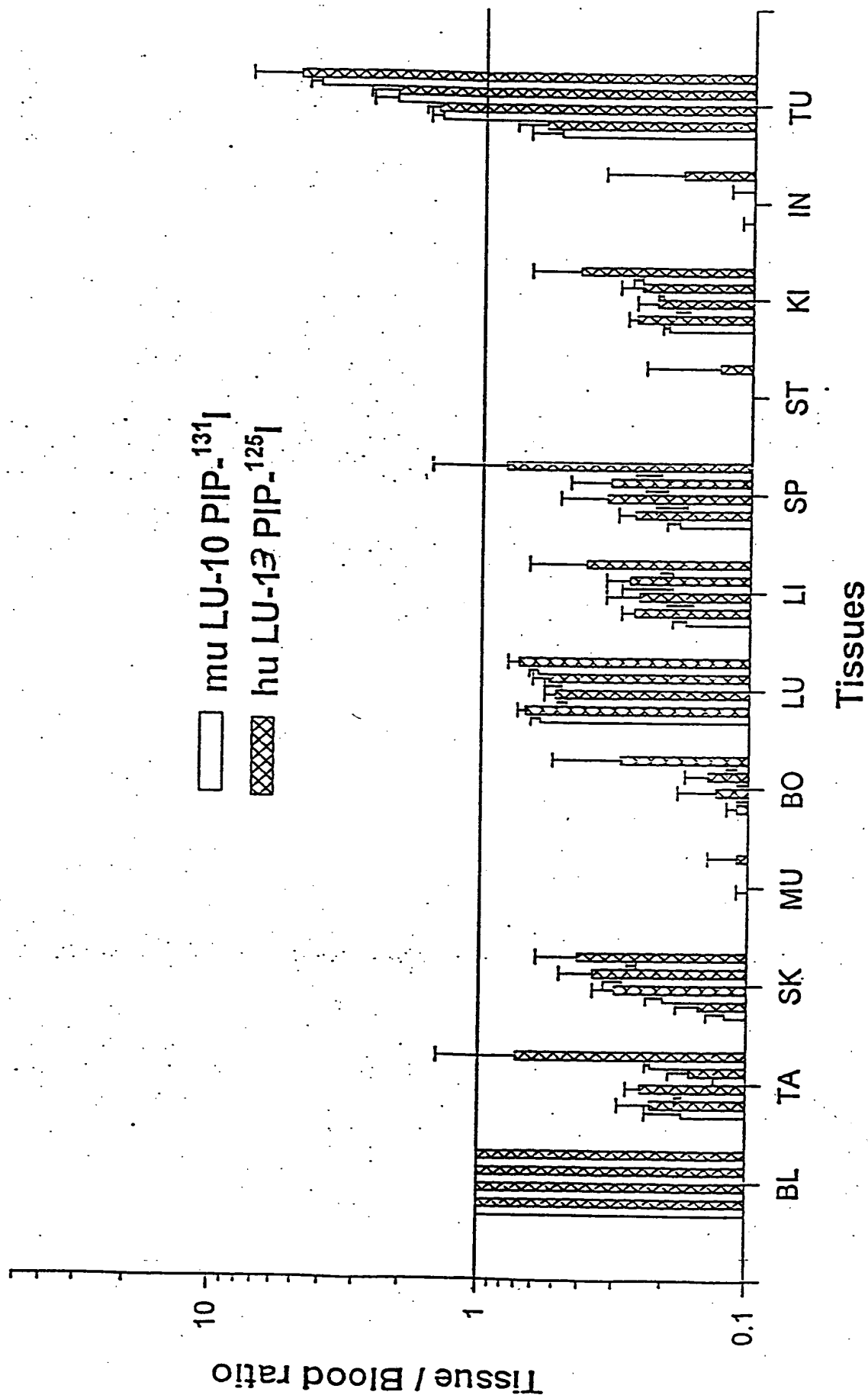


Figure 12

Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTIGEN FOUND BY
ANTIBODY NR-LU-13 AND THEIR USE IN PRETARGETING METHODS

Inventors: Scott S. C. et al. Serial No.: Not yet assigned Docket No.: 690022.527C2

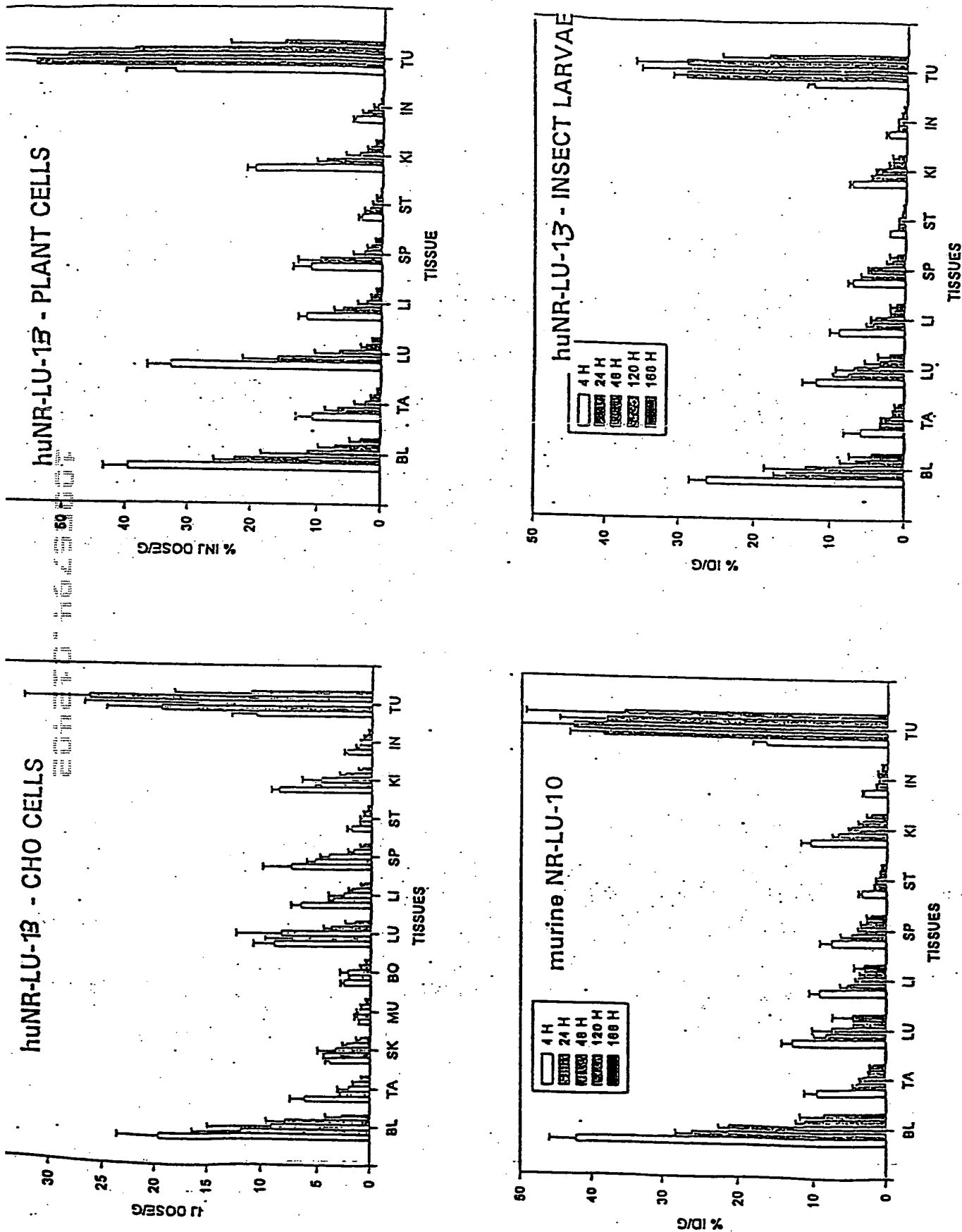


Figure 13

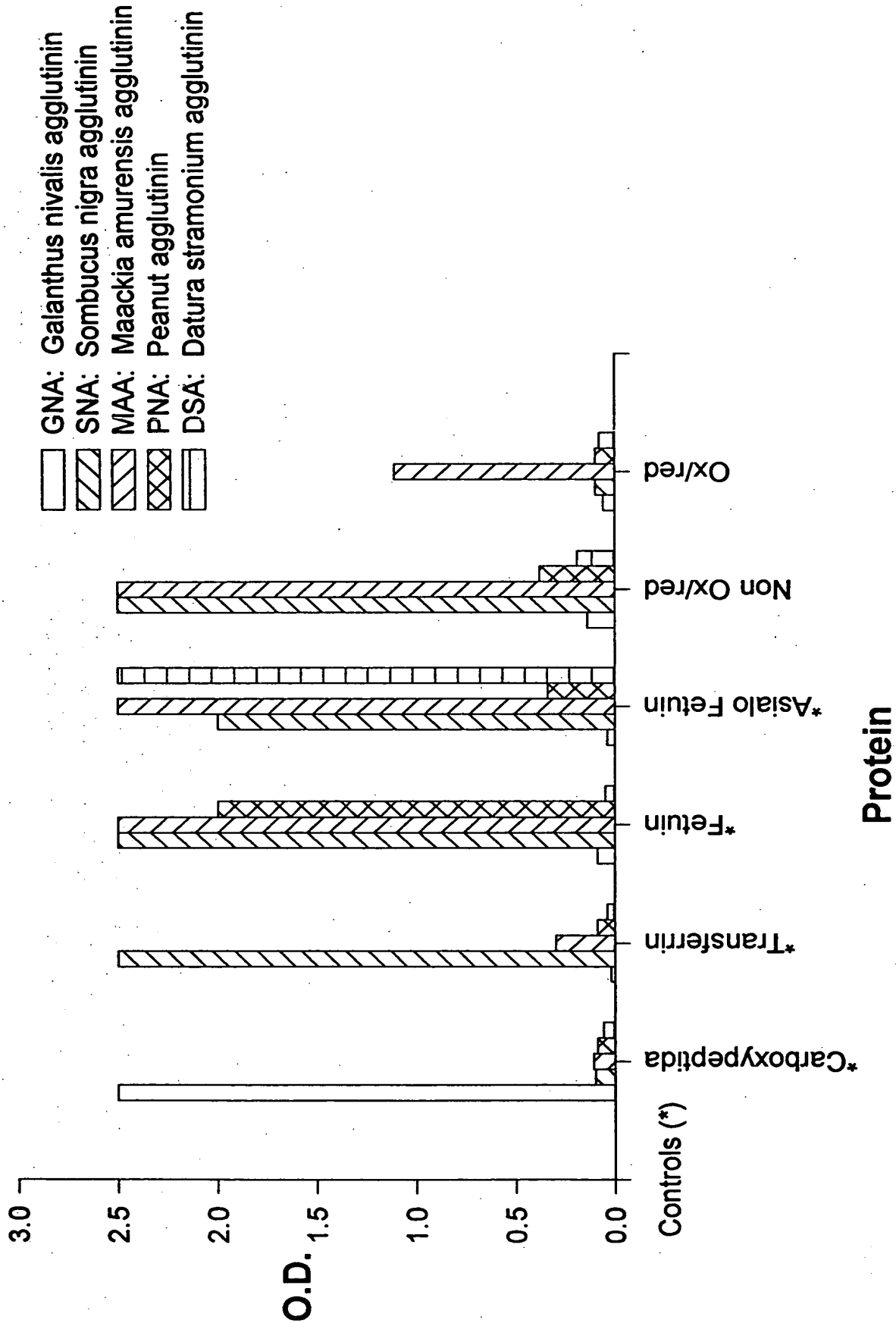


Figure 14

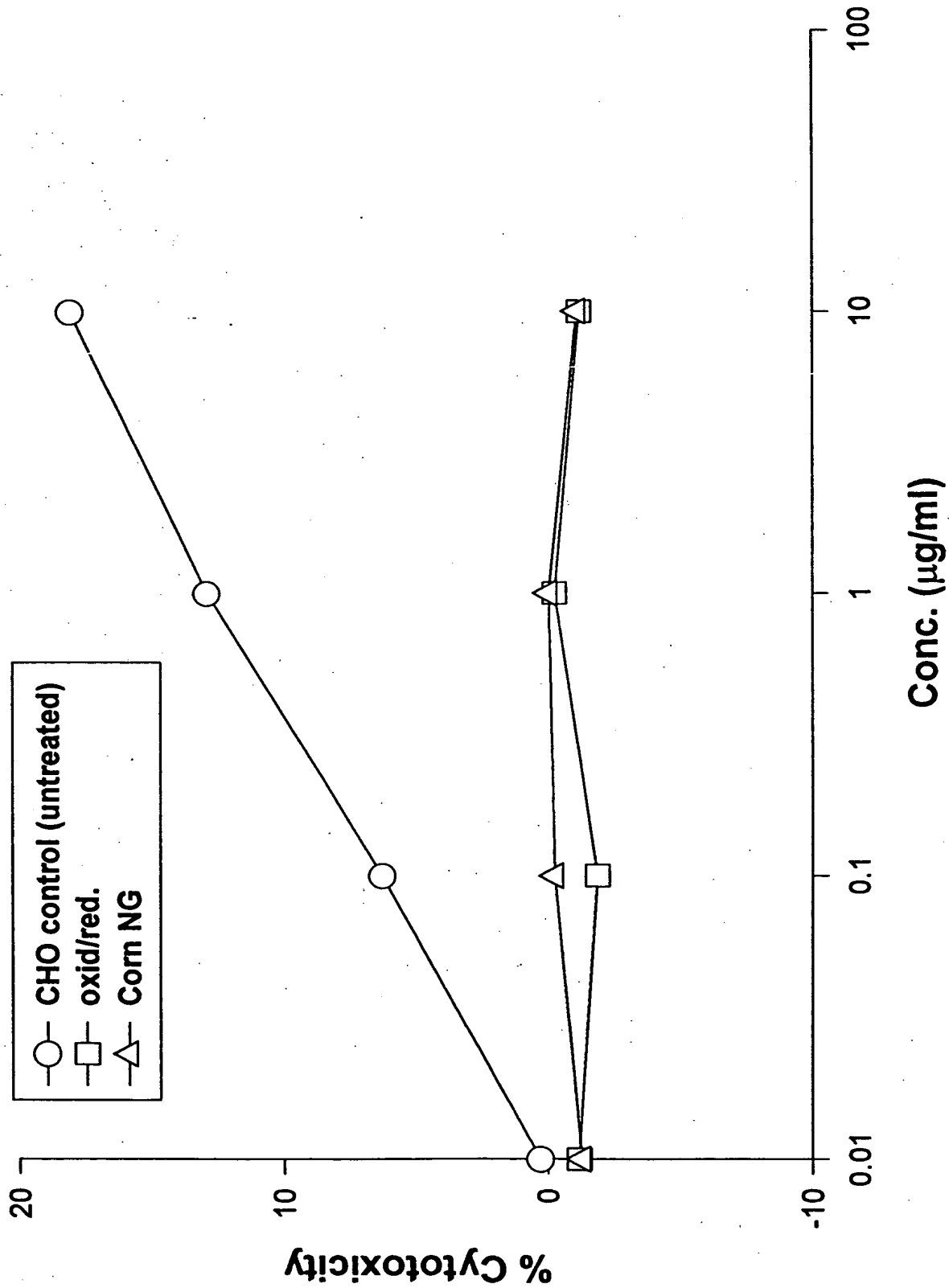


Figure 15a

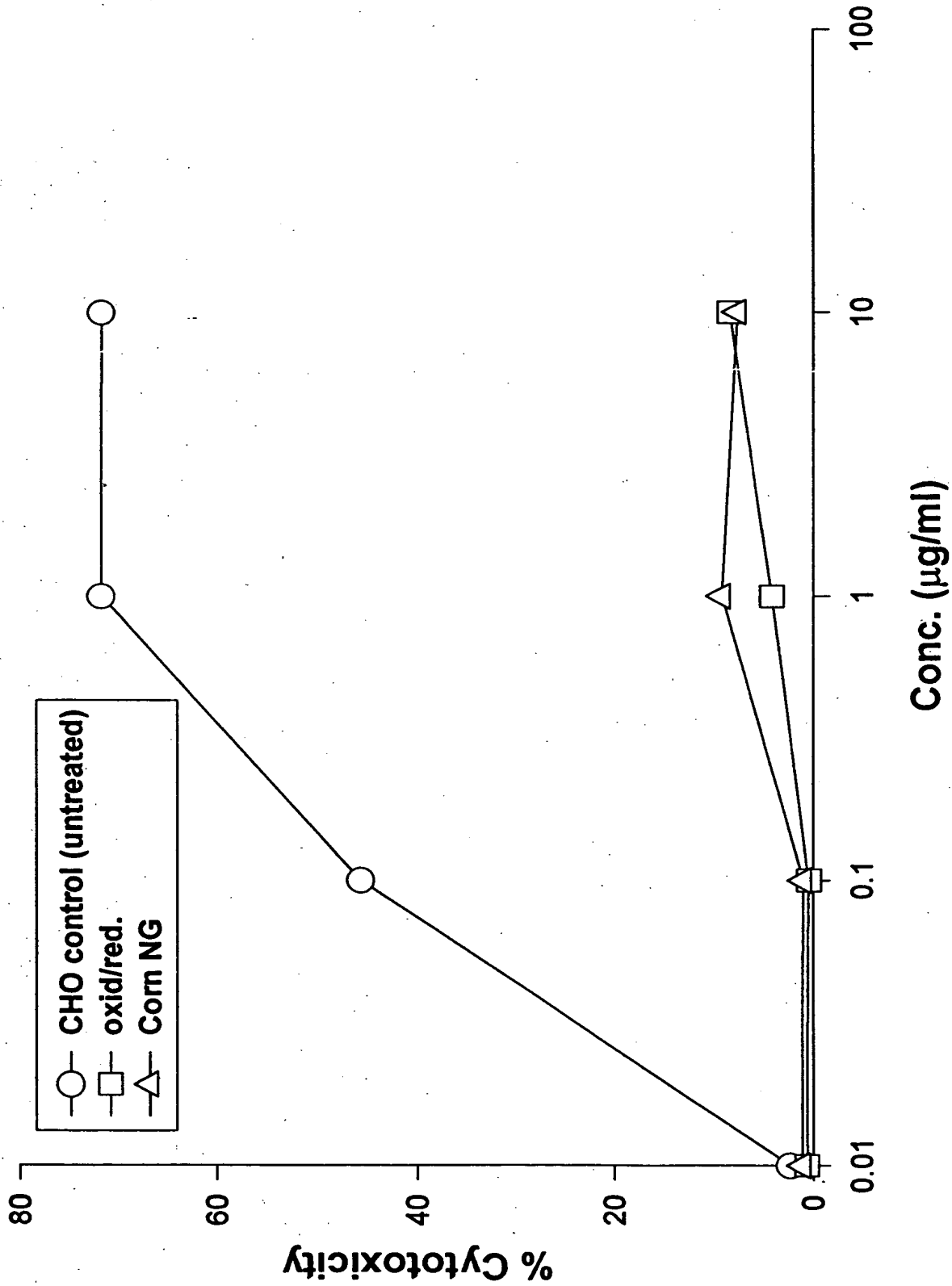


Figure 15b

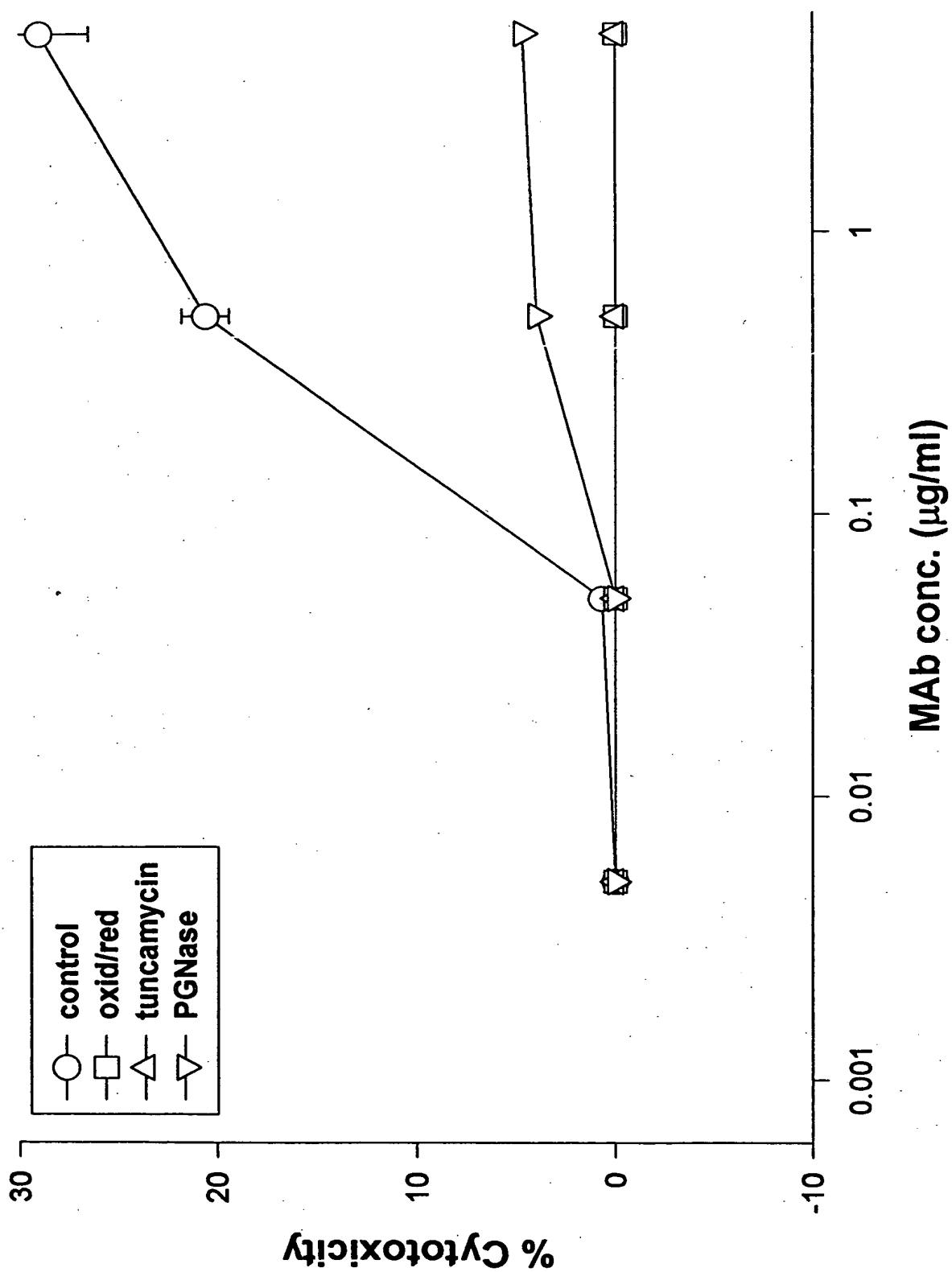


Figure 15c

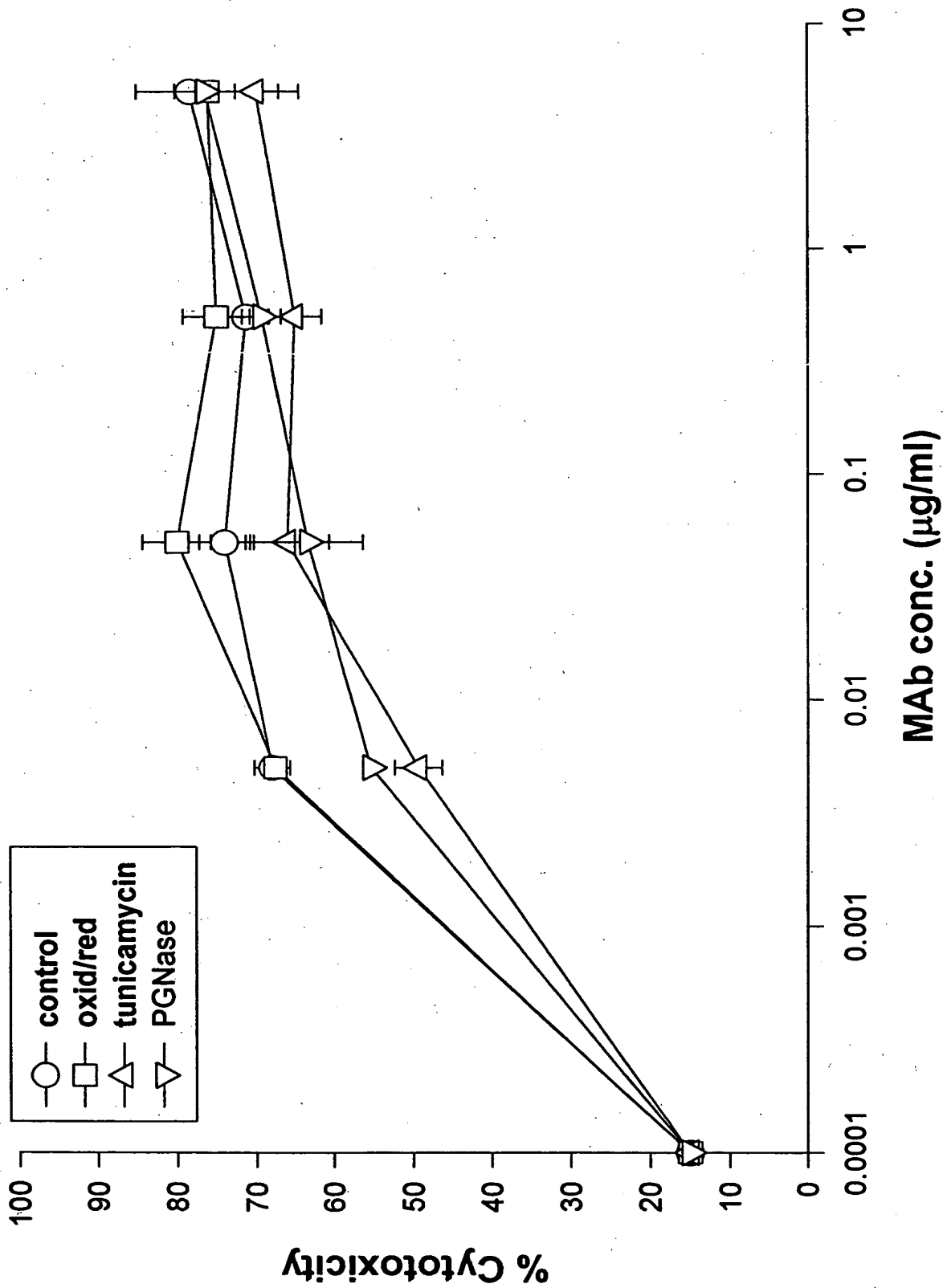


Figure 15d

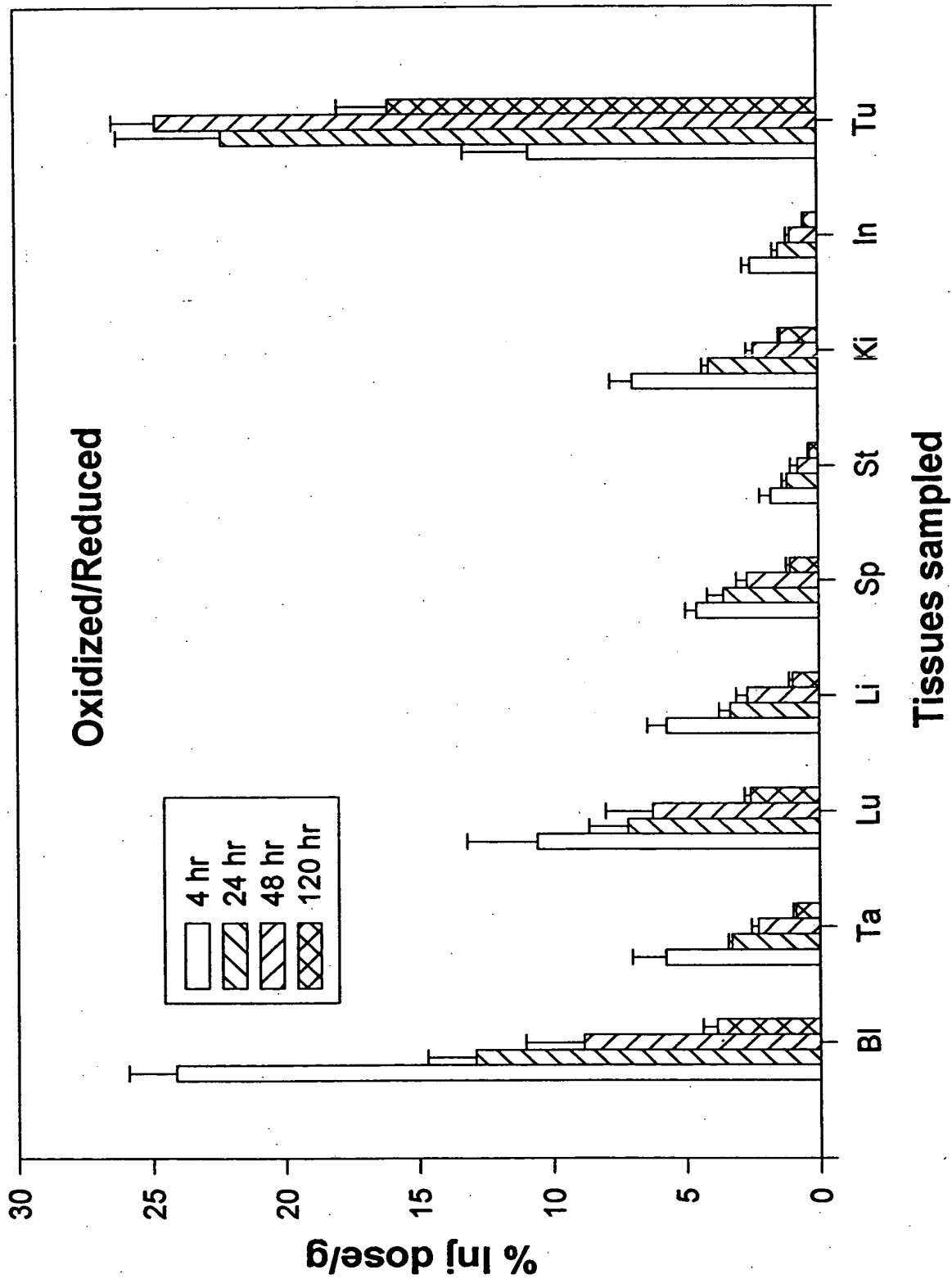


Figure 16a

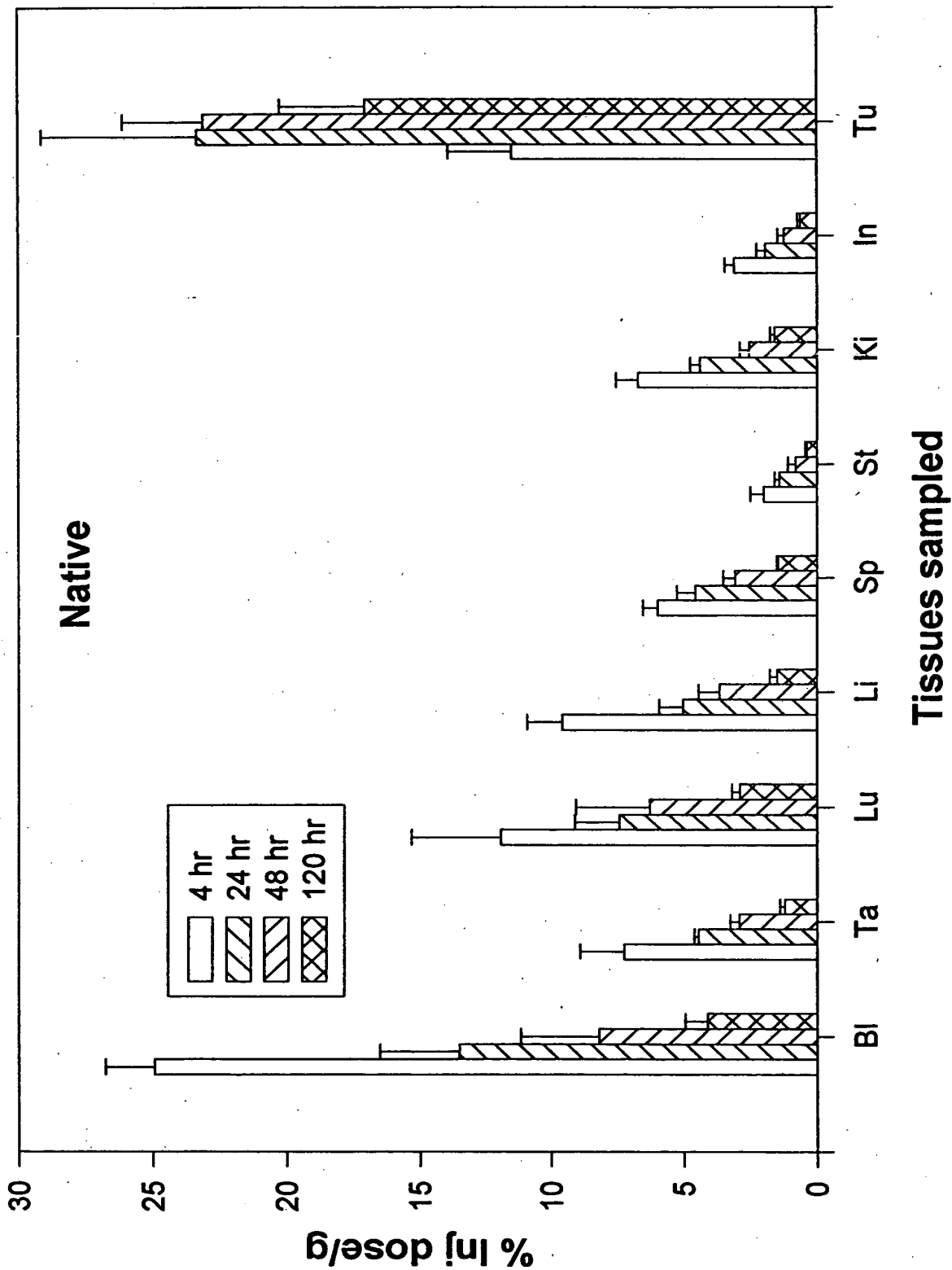


Figure 16b

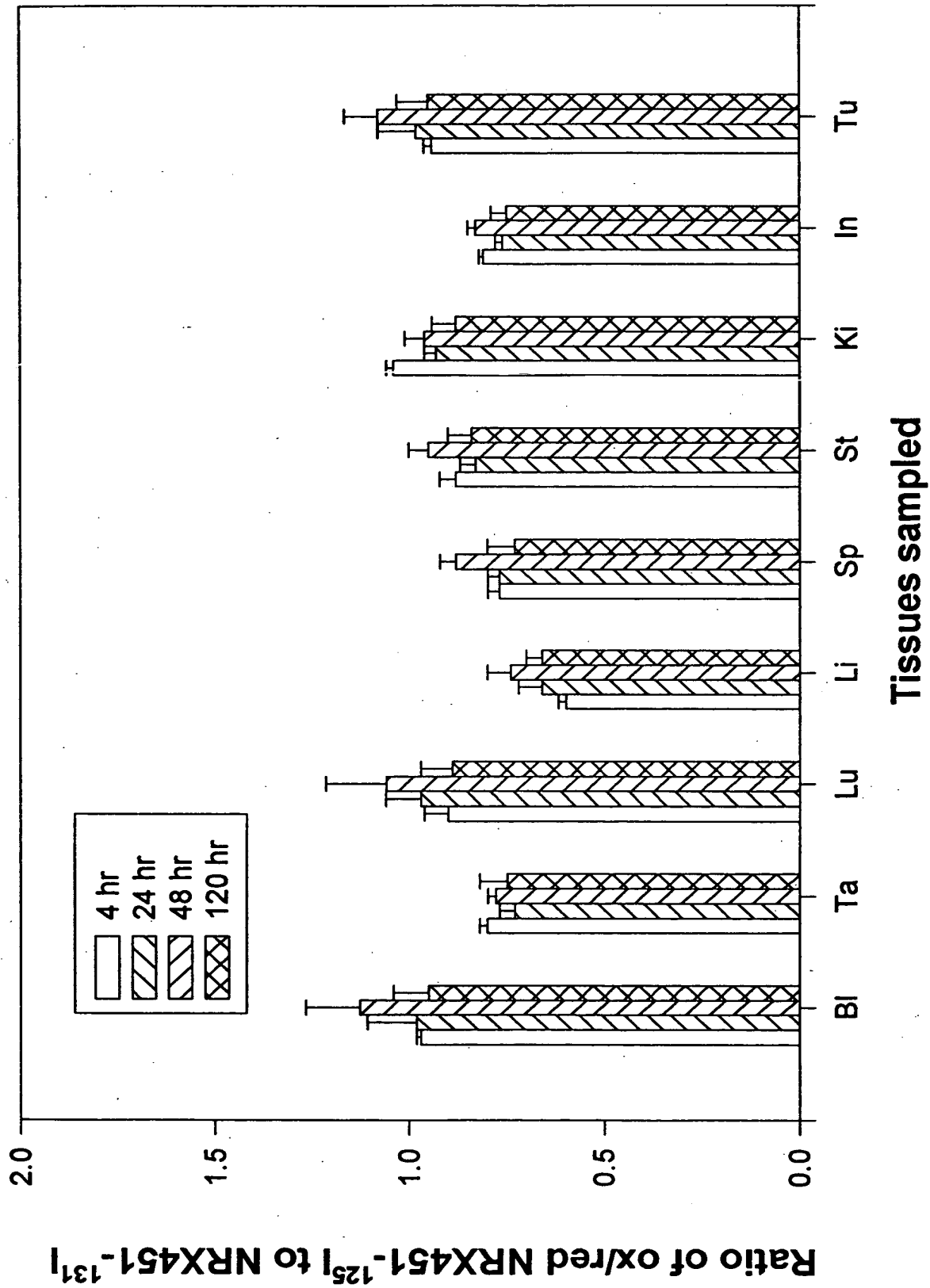


Figure 16c

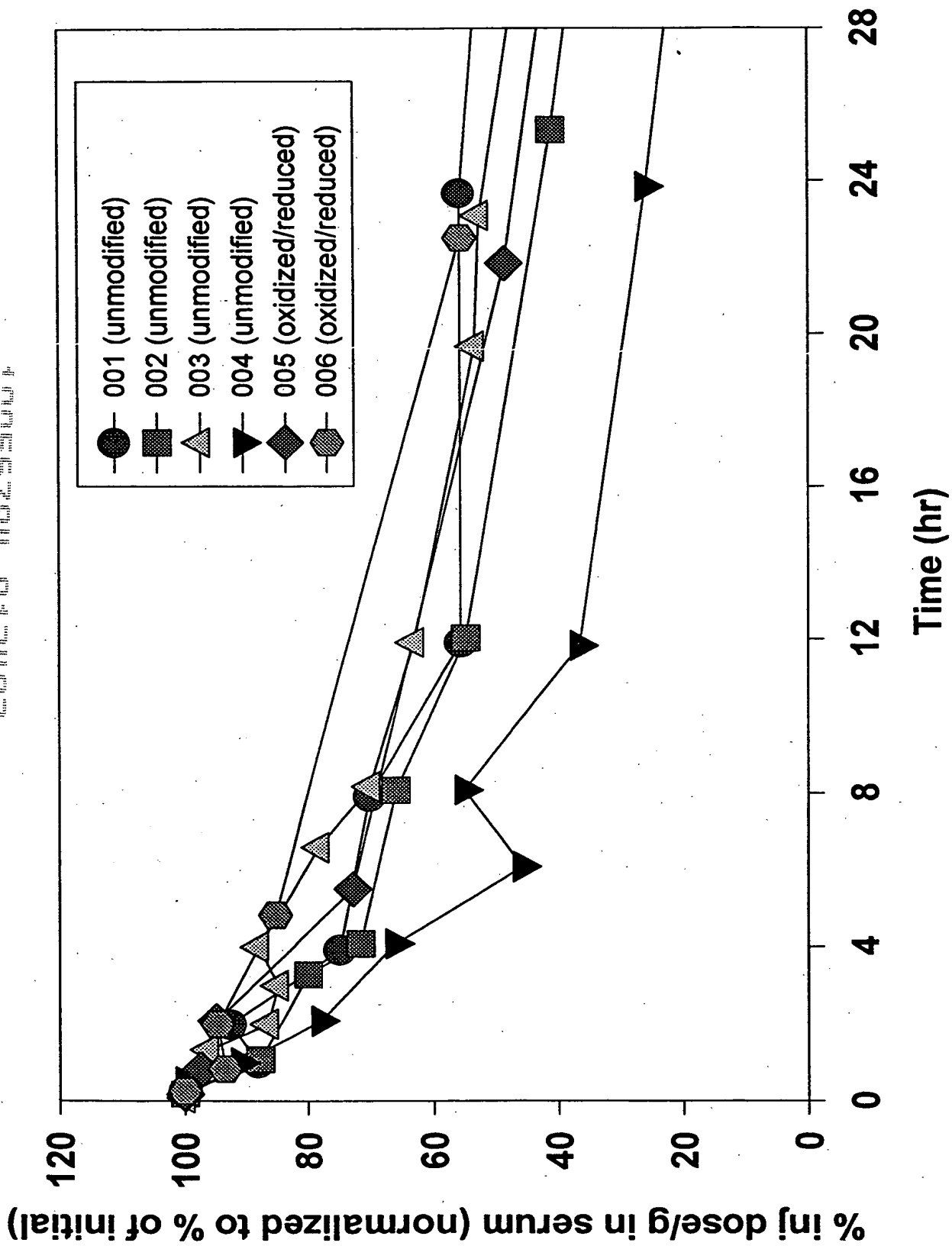


Figure 17

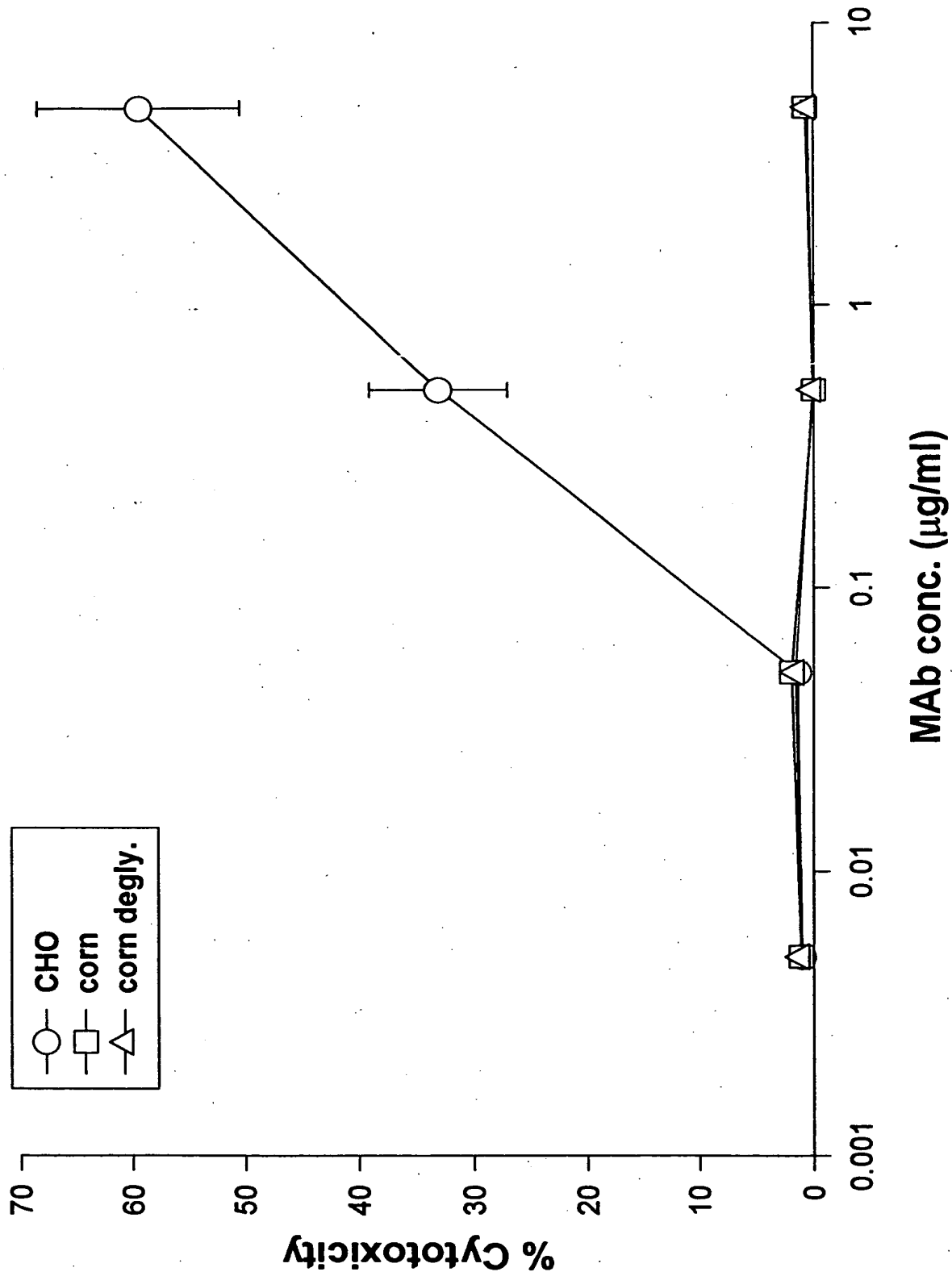


Figure 18

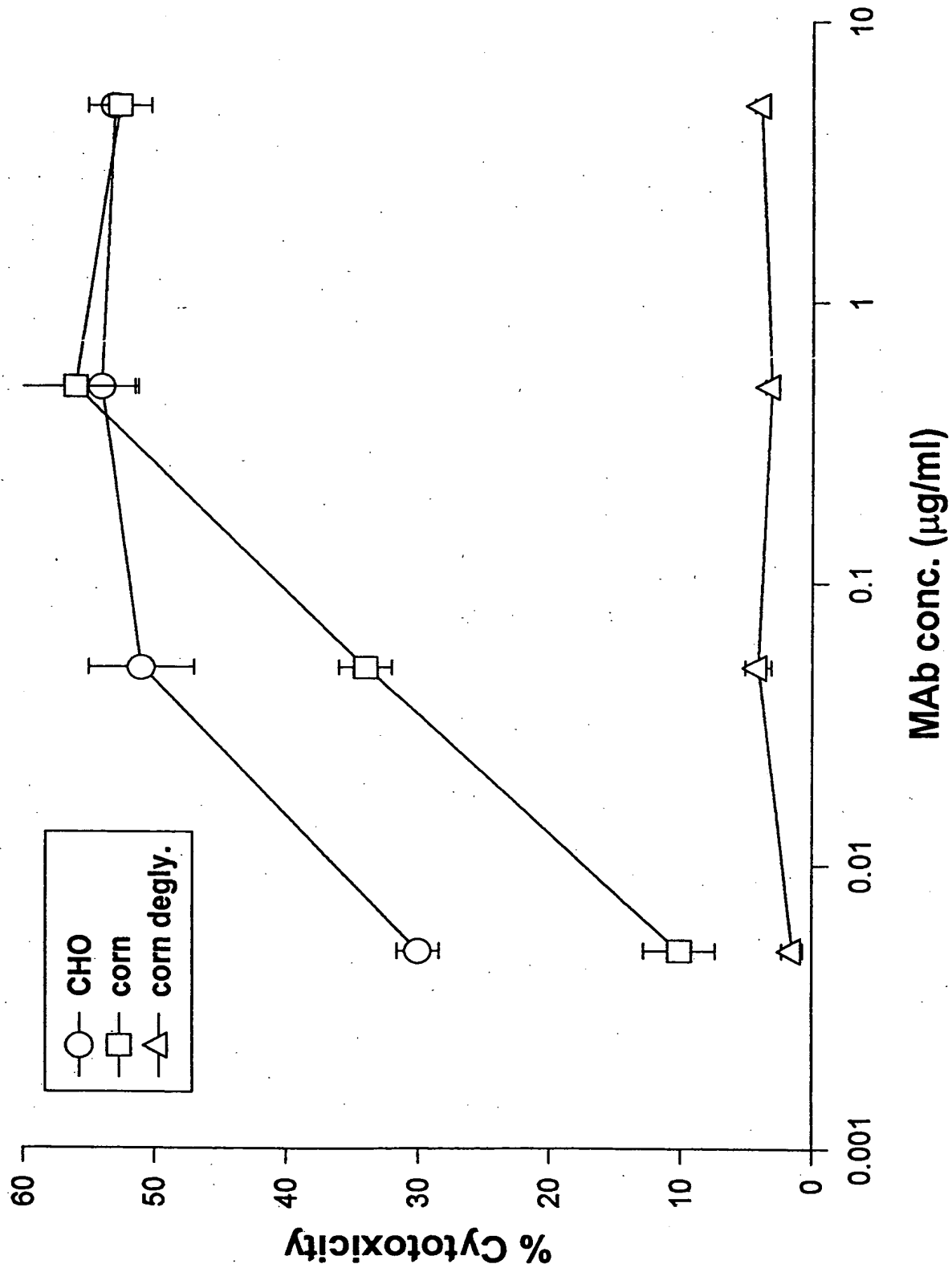


Figure 19

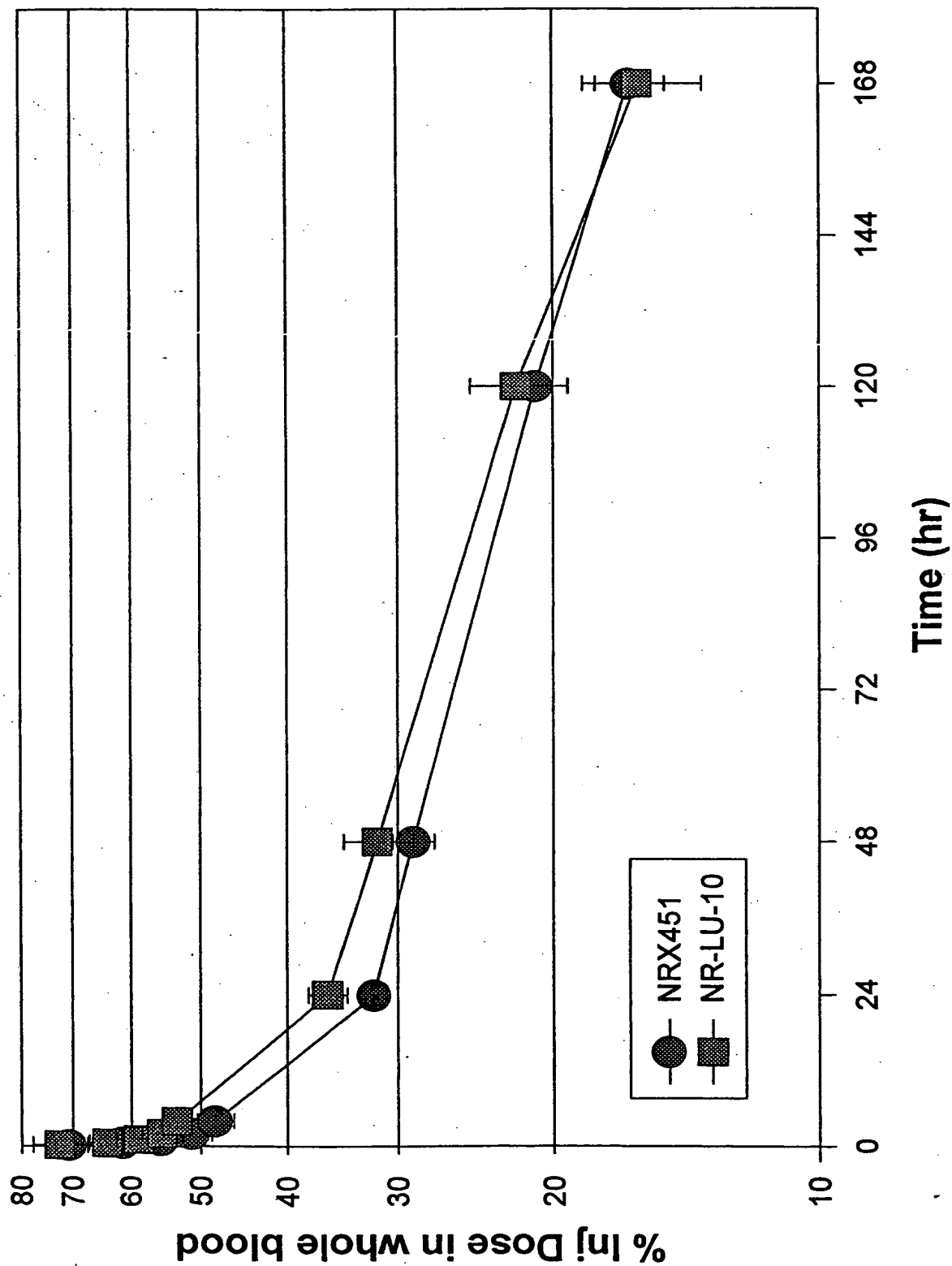


Figure 20

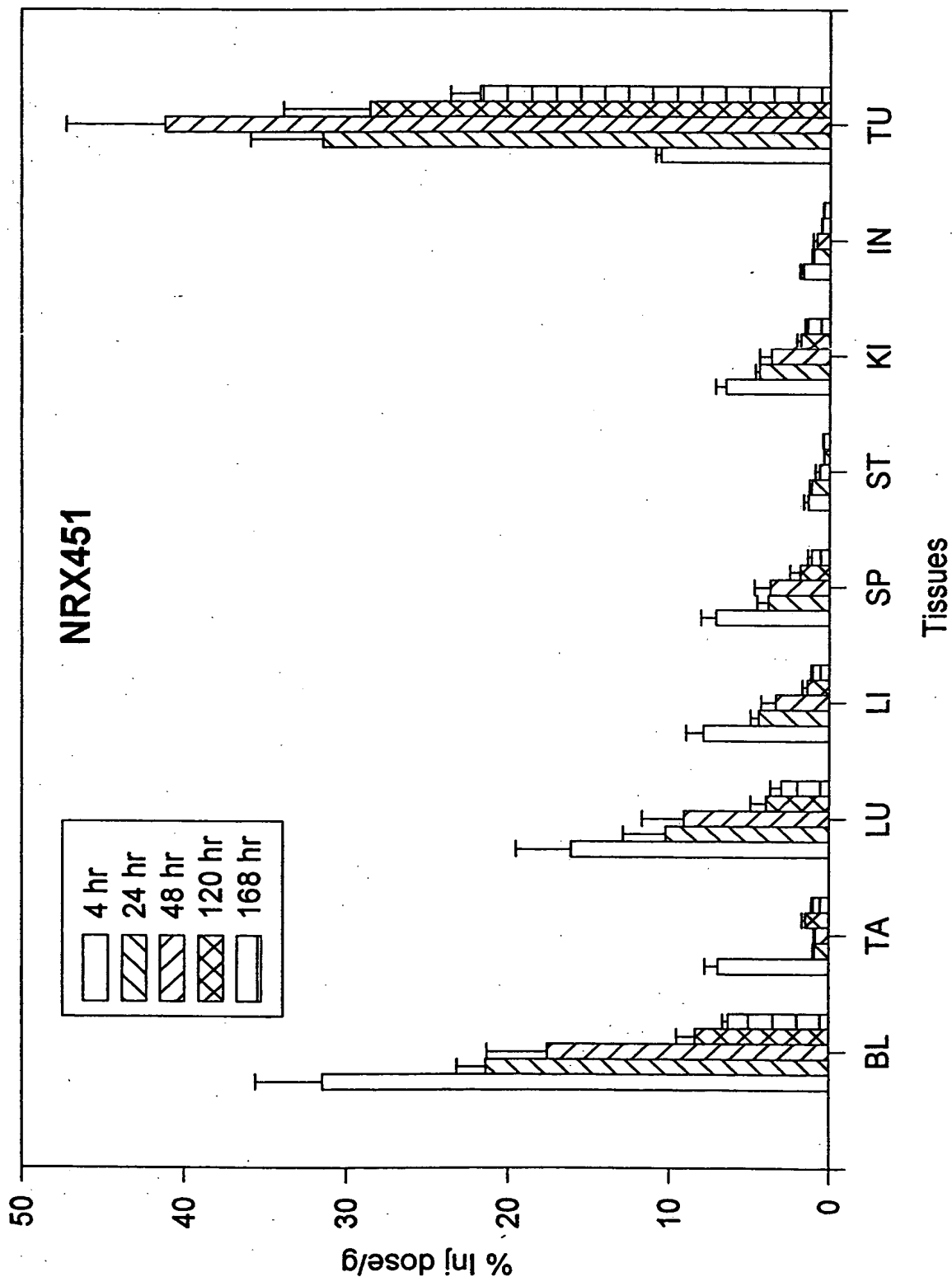


Figure 21a

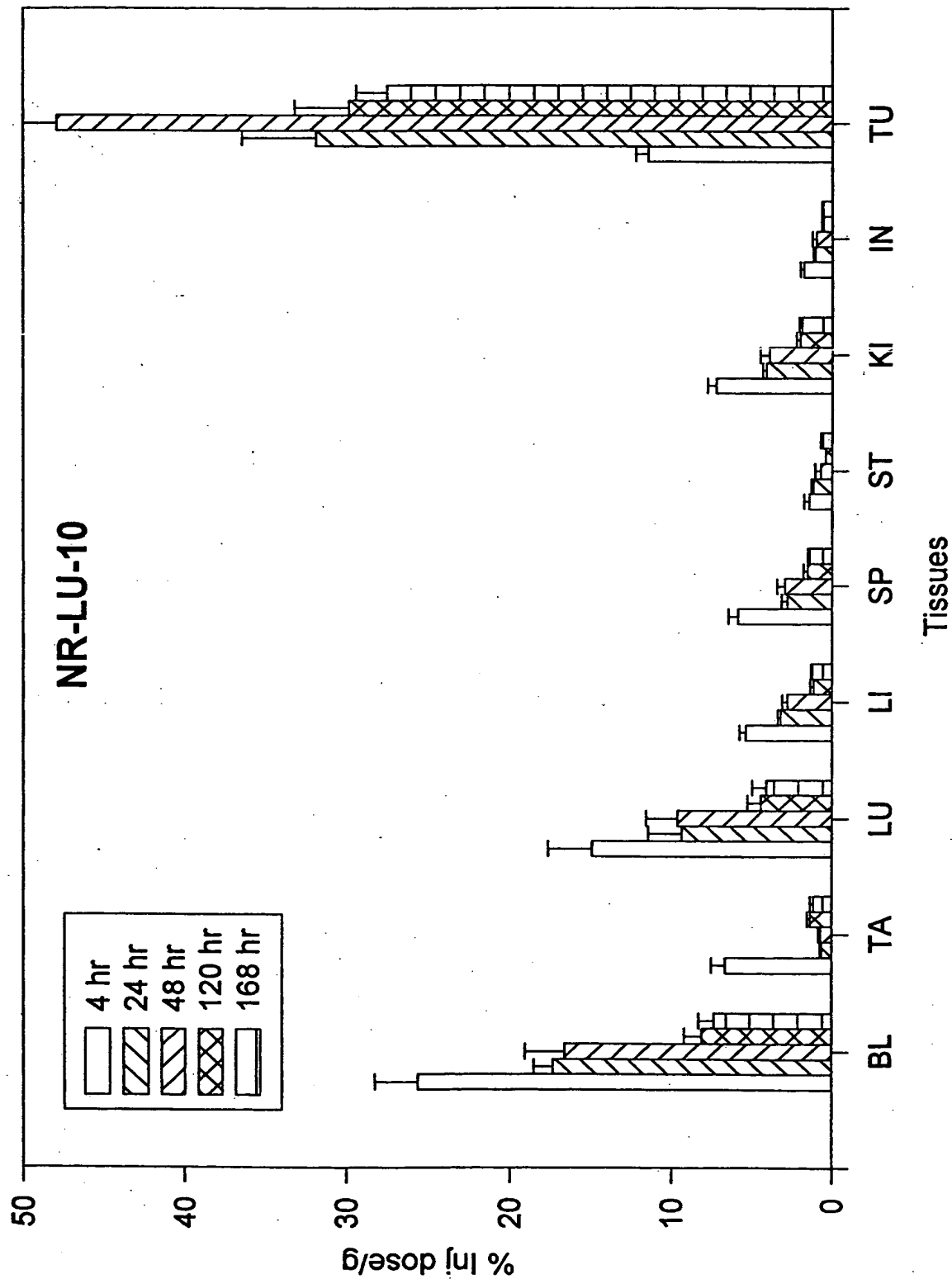


Figure 21b

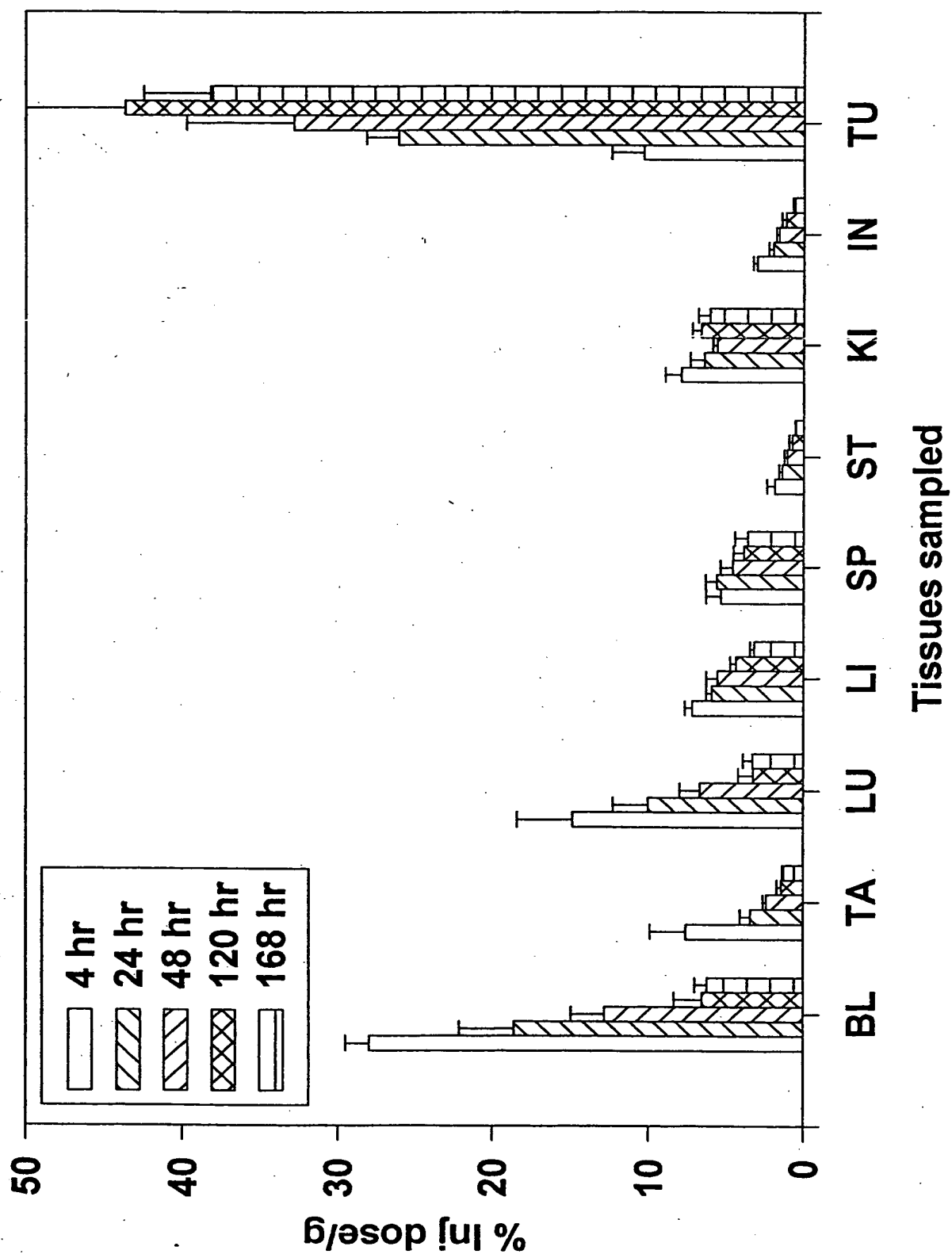


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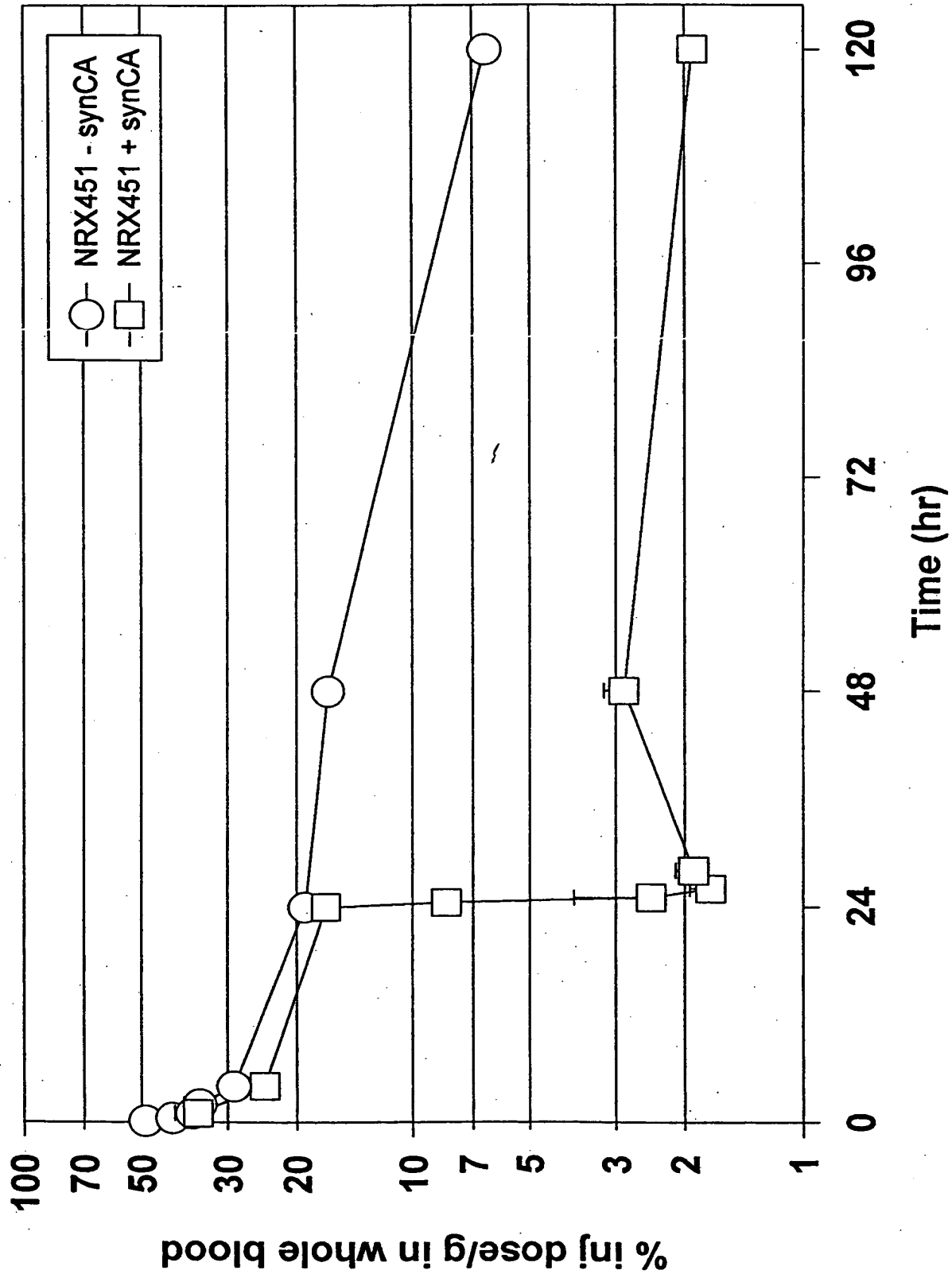


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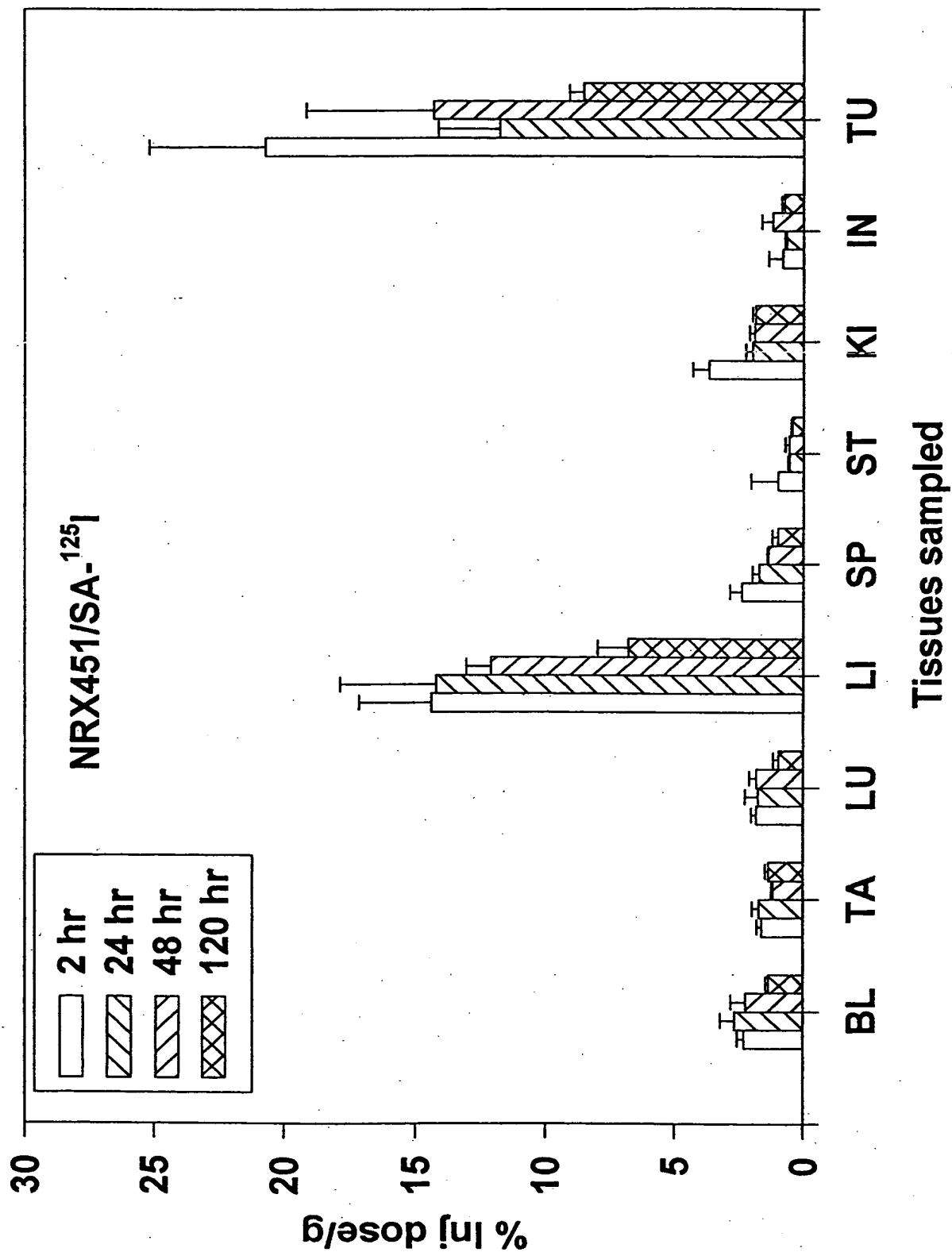


Figure 24a

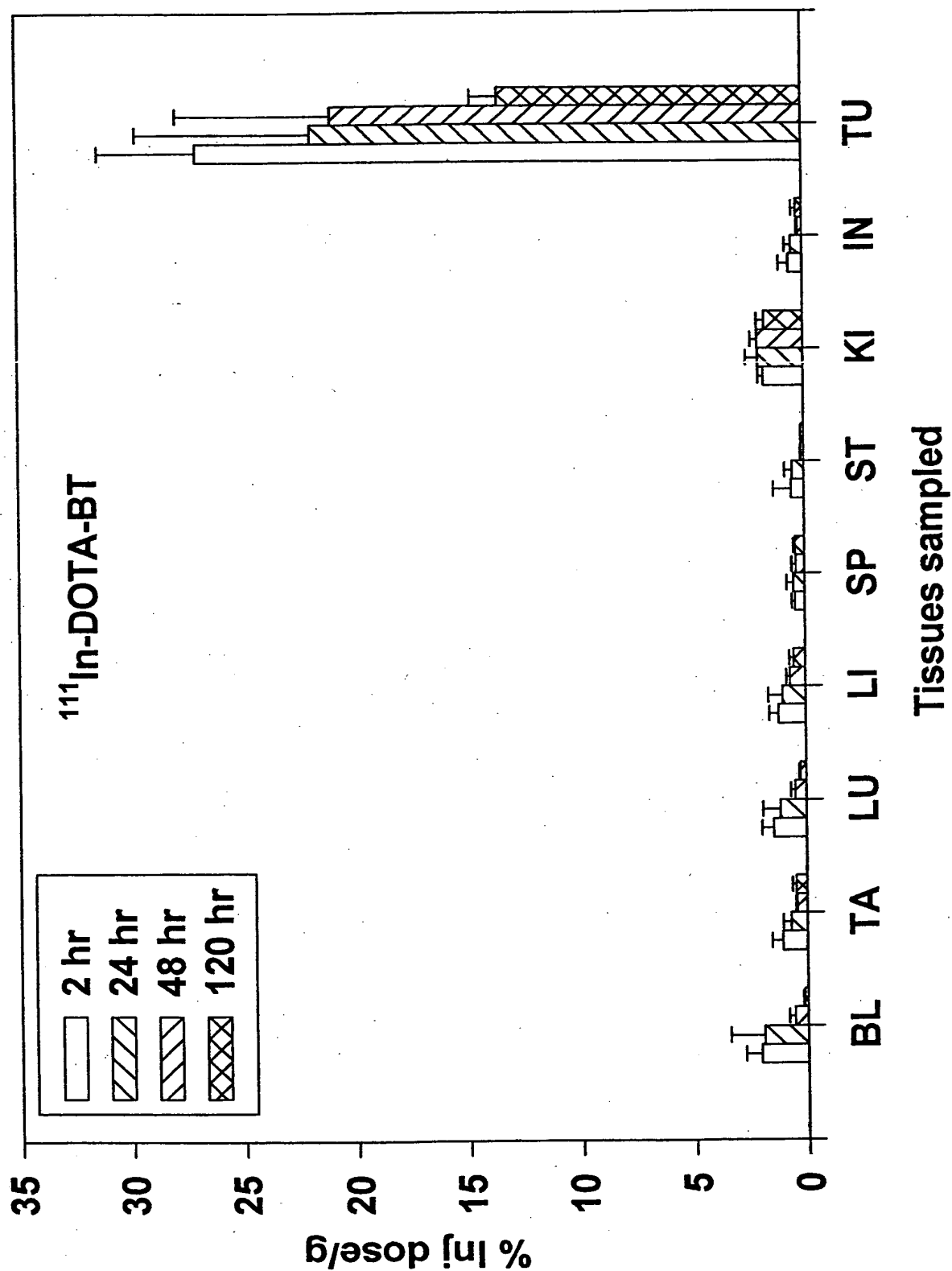


Figure 24b